

Supplemental Material

Supplemental Table S1. SISPA-Seq and reference strain metadata and SISPA-Seq statistics.

Supplemental Table S2. Specific barcoding primer sequences for each strain.

Supplemental Table S3. *In silico* multi-locus sequencing typing (MLST) number of mapped Reads Per Kilobase of gene length per Million total mapped reads (RPKM) values for allelic variants of each loci based on the *A. baumannii* MLST scheme. The top panel highlights the predicted allele based on the highest RPKM value for each loci, along with the predicted sequence type based on the allelic profile. For the ST79 strains, the top *rpoB* allele could not be identified among four variants, of which *rpoB*-5 (the correct one) is one of them.

Supplemental Table S4. Calculated RPKM values for known antibiotic resistance genes and variants from ARG_ANNOT (<http://en.mediterranee-infection.com/article.php?laref=283&titre=arg-annot->).

Table S5. Correspondence between amikacin susceptibility testing and read-based genotyping for two amikacin resistance genes, *armA* and *aphA6*. S: susceptible; R: resistant (>16 µg/uL). MLST RPKM values represent mean, minimum, and maximum of values for the seven MLST loci in each genome. Three true positive strains are included to show representative RPKM and coverage values. ND: not detected

Supplemental Figure S1. Comparison of phylogenies built using SISPA-Seq data subsets. Comparison of phylogeny constructed from SNVs detected using SISPA-Seq reads from A) one primer or B) combined barcoding primers. Branch colors refer to the primary clades in Figure 4.

Supplemental Figure S2. Allele determination using SISPA-Seq data. Identification of top *bla*_{OXA-51}-like allelic variant using BLAST-based RPKM value for example validation strains.